Cadherin 5 is differentially expressed in non-small cell lung cancer and associates with patient survival.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States¹. We mined published microarray data^{2,3,4} to identify differentially expressed genes in NSCLC. We found that the gene encoding cadherin 5 (Cdh5) was among those whose expression was most quantitatively different in human NSCLC tumors as compared to the lung. Cdh5 expression levels were significantly decreased in NSCLC tumors as compared to the lung, and lower expression of Cdh5 in patient tumors was significantly associated with worse overall survival. Cdh5 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: Cdh5, cadherin 5, NSCLC, non-small cell lung cancer, systems biology of NSCLC, targeted therapeutics in NSCLC.

1 In 2016, lung cancer resulted in the death of 158,000 Americans; 81% of all patients
2 diagnosed with lung cancer will expire within 5 years⁵. Non-small cell lung cancer (NSCLC) is
3 the most common type of lung cancer, diagnosed in 84% of patients with lung cancer, and 76%
4 of all patients with NSCLC will expire within 5 years⁵. The rational development of targeted
5 therapeutics to treat patients with NSCLC can be supported by an enhanced understanding of
6 fundamental transcriptional features of NSCLC tumors. To discover genes associated with
7 NSCLC tumors in an unbiased fashion and at the systems-level, we mined independently
8 published microarray data^{2,3,4} to compare global gene expression profiles of NSCLC tumors to
9 that of the normal lung. We found recurrent and significant differential expression of the gene
10 encoding cadherin 5 (Cdh5), also known as vascular endothelial cadherin (VE-cadherin) in
11 adenocarcinoma tumors from patients with NSCLC, suggesting Cdh5 may be important for
12 NSCLC tumor initiation or progression.

13 **Methods**

14 We utilized microarray datasets, GSE43458², GSE33532⁴ and GSE74706⁴ for this
15 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE43458
16 was generated using Affymetrix Human Gene 1.0 ST Array technology; for this analysis, we
17 used with $n=30$ control lung tissue and $n=80$ NSCLC tumors, and the analysis was performed
18 using platform GPL6244. GSE33532 was generated using Affymetrix Human Genome U133
19 Plus 2.0 Array technology; for this analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC
20 tumors, and the analysis was performed using platform GPL570. GSE74706 was generated
21 using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for this

analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was performed using platform GPL13497. All tumors utilized for differential gene expression analysis here were of the adenocarcinoma type.

The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether Cdh5 expression was significantly between normal lung tissue and NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of Cdh5 mRNA expression levels with overall survival in $n=1925$ non-small cell lung cancer patients.

Results

We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to discover in an unbiased fashion and at the transcriptome-level the most striking gene expression features of NSCLC tumors.

Cdh5 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding cadherin 5 (Cdh5), also known as vascular endothelial cadherin (VE-cadherin) in NSCLC tumors when compared to the

lung² (Table 1). When sorting each of the transcripts measured based on significance of difference in expression of Cdh5 between NSCLC tumors and the normal lung, in this dataset, Cdh5 ranked 59 out of 25906 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of Cdh5 in NSCLC tumors was statistically significant (Table 1; $p=9.37E-26$).

We queried a second microarray dataset³ to determine if we could validate differential expression of Cdh5 in non-small cell lung cancers (Table 2). We found significant differential expression of Cdh5 in NSCLC tumors of the adenocarcinoma type when compared to the normal lung (Table 2). When sorting each of the transcripts measured based on significance of difference in expression of Cdh5 between NSCLC tumors and the normal lung, Cdh5 ranked 92 of 33252 total transcripts (Table 2), equating to 99.7% differential expression. Differential expression of Cdh5 in NSCLC tumors was statistically significant (Table 2; $p=4.54E-15$).

Analysis of a third microarray dataset⁴ again revealed significant differential expression of Cdh5 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts measured based on significance of difference in expression of Cdh5 between NSCLC tumors and the normal lung, Cdh5 ranked 597 out of 34183 total transcripts (Table 3), equating to 98.3% differential expression. Differential expression of Cdh5 in NSCLC tumors was statistically significant (Table 3; $p=3.25E-09$).

Cdh5 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.

We obtained exact mRNA levels for Cdh5 from NSCLC tumors and from the lung to

1 directly compare Cdh5 expression between tumor and control lung tissue and assess for
2 statistical significance. Cdh5 was expressed at significantly lower levels in NSCLC tumors as
3 compared to the normal lung in each dataset queried, respectively (Figure 1: $p < 0.0001$ and
4 Figure 2: $p < 0.0001$). We calculated a mean fold change of 0.8141 ± 0.0721 and 0.7650 ± 0.0598
5 in Cdh5 expression when comparing NSCLC tumors to the lung (Table 1 and Table 2,
6 respectively).

7 Cdh5 expression in NSCLC tumors correlates with overall survival.

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10 We performed Kaplan-Meier survival analysis using Cdh5 mRNA expression in NSCLC
11 tumors coupled with paired overall survival data from each patient, in 1925 NSCLC patients in
12 total, to determine whether Cdh5 tumor expression was correlated with survival outcomes in
13 NSCLC. We found that patients whose tumors expressed lower levels of Cdh5 possessed
14 significantly shorter overall survival than patients with high tumor expression of Cdh5 (Figure
15 3). Median overall survival (OS) of patients in the low expression cohort was 56.5 months,
16 while median OS in patients in the high Cdh5 expression cohort was 98.5 months (Table 4); this
17 difference in median OS based on Cdh5 tumor expression in NSCLC was statistically significant
18 (Figure 3; logrank p -value: $7.5e-9$; hazard ratio: 0.65 (0.56 - 0.75); false discovery rate=0.01).
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24 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
25 differential expression of cadherin 5 as among the most significant transcriptional features of
26 NSCLC tumors, and Cdh5 expression was significantly correlated with patient outcomes, as
27 patients with lower tumor expression of Cdh5 possessed significantly worse overall survival.
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Discussion

Cadherin 5, also known as vascular endothelial (VE) VE-cadherin, was originally identified as an endothelial cell-specific membrane protein based on antibody staining of intercellular contacts by the 7B4 monoclonal antibody⁷. Cadherin 5 is a transmembrane protein that spans 784 amino acids^{8,9}. Ectopic expression of cadherin 5 in Chinese hamster ovary (CHO) cells results in its localization at cell-cell contacts, and in endothelial cells, cadherin 5 can co-immunoprecipitate with α -catenin⁸. Cadherin 5 can function in cell-cell adhesion through homophilic calcium-dependent interactions. In a cell monolayer assay *in vitro*, expression of cadherin 5 results in 40-50% decrease in permeability on exposure to high molecular weight molecules like immunoglobulins⁸. Another phenotypic consequence of cadherin 5 expression in CHO cells is impairment of cellular proliferation. Cadherin 5 influence on cell proliferation is in part due to interactions with catenins, as expression of a cadherin 5 variant that lacks the cytoplasmic domain responsible for catenin interaction fails to inhibit cellular proliferation⁹. However, the amino-terminal extracellular domain was also important for this phenotype, suggesting that both interactions between cadherin 5 and catenins, as well as extracellular homophilic interactions are necessary for cadherin 5-mediated inhibition of cellular proliferation⁹. In conjunction with β -catenin, F-actin, and CD31, cadherin 5 can also function in the formation of endothelial cell tubes¹⁰. Changes in cellular confluence can influence vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) activation in response to VEGF; cadherin 5 antibody blockade enhances VEGFR2 activation in response to VEGF, suggesting

cadherin 5 is important in this context¹¹. In mice, cadherin 5 is important for endothelial cell survival: truncation of cadherin 5 in its β -catenin-binding domain results in lethality at embryonic day 9.5 (E9.5), with endothelial cell death (apoptosis) due to failure of VEGF-A to induce survival signaling via Bcl2 and the protein kinase Akt¹². Intraperitoneal injection of a rat monoclonal antibody targeting cadherin 5 (VECD1) results in death associated with subcutaneous hemorrhage and venostasis, providing evidence that in mammals cadherin 5 function supports vascular integrity¹³. A function of cadherin 5, at adherens junctions, that supports control of junctional permeability is transcriptional induction of claudin-5 expression at tight junctions through FoxO1 and Tcf4; cadherin 5 can function through the protein kinase Akt to induce FoxO1 phosphorylation to control β -catenin nuclear translocation¹⁴. Another mechanism by which cadherin 5 is involved in changes in vascular permeability is endocytosis of cadherin 5 in response to VEGF stimulation, involving VEGFR2 and Vav-mediated activation of Rac, leading to recruitment of β -arrestin2 following VEGFR2 phosphorylation by p21-activated kinase¹⁵.

Dysregulated expression of cadherin 5 has been implicated in a number of human cancers. Cadherin 5 has been described as a biomarker in metastatic breast cancer¹⁶, and in gastric cancer, high expression of cadherin 5 was correlated with invasion of the venous and lymphatic systems, metastasis to the lymph nodes and to distant sites, as well as worse patient survival - patients with high cadherin 5 expression possessed significantly worse disease-specific survival¹⁷. In human lung cancer cell lines, expression of cadherin 5 at the protein level is lost in

several (but not all) cell lines, including H322, H460, H441 and H2228¹⁸. At the RNA level, expression of cadherin 5 appeared to segregate with EGFR mutation status, with wild-type EGFR cell lines (e.g., A549) expressing markedly less cadherin 5 mRNA as compared to EGFR mutant cell lines (e.g., PC9 and H1975)¹⁸. Treatment of PC9 and H1975 cell lines with Afatinib, an EGFR tyrosine kinase inhibitor, resulted in decreased expression of cadherin 5 at the protein level¹⁸. A549 non-small cell lung cancer (NSCLC) cells expressing exon 19 deletion in EGFR, which was associated with increased cadherin 5 expression, displayed significantly increased migration speed and invasion number *in vitro* as compared to control A549 cells, suggesting that EGFR mutation status could function at least in part through changes in cadherin 5 expression to enhance cancer cell migration and invasion in lung cancer¹⁸. We identified one study which supported findings from our analysis, describing decreased and differential expression of cadherin 5 in patients with NSCLC¹⁹. Thus, cadherin 5 can function in to support vascular and junctional integrity, can inhibit cellular proliferation, can control endothelial cell survival, and its expression is perturbed in a number of human cancers.

We found, by mining multiple independently published microarray datasets, that Cdh5 was among the genes most differentially expressed in the primary tumors of patients with non-small cell lung cancer; Cdh5 was expressed at significantly lower levels in NSCLC tumors as compared to the lung, and decreased expression of Cdh5 was significantly associated with worse overall survival in NSCLC patients. Cdh5 may be of relevance to the biology of tumor initiation or progression in patients with NSCLC of the adenocarcinoma type, the most common type of the leading cause of cancer death in the United States and worldwide.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
59	7996264	9.37E-26	-13.757043	48.033906	0.8141 ± 0.0721	CDH5	cadherin 5

Table 1: Cdh5 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, fold change of Cdh5 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
92	204677_at	4.54E-15	-14.124508	24.3902251	0.7650 ± 0.0598	CDH5	cadherin 5

Table 2: Cdh5 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
597	A_33_P3728167	3.25E-09	-8.37	11.1220083	CDH5

Table 3: Cdh5 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, fold change of Cdh5 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Figure 1: Cdh5 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of Cdh5 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in Cdh5 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: Cdh5 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of Cdh5 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in Cdh5 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: Cdh5 expression in NSCLC tumors significantly correlates with overall survival.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1925$ total patients stratified into two groups, based on low or high expression of Cdh5 in patient tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on Cdh5 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
56.5	98.5

Table 4: Cdh5 expression in NSCLC tumors significantly correlates with patient survival.

The median overall survival of $n=1925$ NSCLC patients based on stratification into low or high expression of Cdh5 in tumors is listed in this chart.